Experiments on the Kidneys of the Frog. (Preliminary Communication.)

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(Communicated by Prof. C. J. Martin, F.R.S. Received March 27,—Read April 24, 1913.)

Introduction.

As is well known, the glomeruli of the frog's kidney are supplied with blood only by the renal arteries, whereas the renal tubules have a double supply. On the one hand, they receive blood by way of the renal portal veins; on the other hand, the efferent vessels from the glomeruli open into the capillary network round the tubules. The whole of the tubule receives blood from each of these two sources, so that the capillary network around the tubules can be fully injected either from the renal arteries or from the renal portal veins. Taking advantage of this fact it has been shown by Beddard and one of us (F. A. B.) that after complete occlusion of the glomeruli the tubules, when adequately supplied with oxygen, maintain their normal histological appearance, and may secrete urine. In the present experiments an attempt has been made to determine the function of the glomerulus and to ascertain whether the tubules possess the capacity to absorb water and solids.

Methods.

1. *Experimental.*—All the experiments were carried out on fully pithed frogs. In the earlier experiments the following method was adopted:—Ligatures were tied round the fore limbs, the heart was exposed, and the right aortic arch tied off. The ventricle and auricles were freely opened, and a cannula connected with a perfusion bottle was tied into the left aortic arch. The arterial perfusion was started at once, and the ventricle and auricles were then excised. This procedure was carried out as quickly as possible after the frog was pithed, and usually took five or six minutes. It is of importance to commence the arterial perfusion at the earliest possible moment. When most of the blood was washed out of the circulation the anterior abdominal vein was tied in two places and divided, the hind legs were tied off, and a cannula was placed in the inferior end of the anterior abdominal vein and connected with a perfusion bottle. The fluid leaving the kidneys was collected by means of a cannula placed in the beginning of the vena cava just beyond its origin from the renal veins. Finally, cannulæ were placed in the ureters. This latter operation was

much more easily carried out in the male than in the female frog, and the former were almost invariably used. Frequently the mesenteric artery was also ligatured in order to limit the extent of the perfusion.

In the later experiments the aortæ were exposed immediately above the kidneys, the right was tied off, and a cannula was placed in the left; the mesenteric artery was ligatured. Cannulæ were then placed in the vena cava just above the renal veins, the inferior end of the anterior abdominal vein (after tying off the legs) and the ureters. The testes were removed by the cautery. The advantage of this method is that the perfusion is practically confined to the kidneys, and the arterial pressure can be more readily gauged and adjusted than in the earlier experiments. The arterial perfusion through the aorta was made at a pressure of 20–24 cm. of water; the venous perfusion pressure varied from 10 to 14 cm. of water.

Solutions Used.—The following perfusing fluids were used: (1) Normal Ringer's solution (NaCl 0·65 per cent., KCl 0·02 per cent., $CaCl_2$ 0·03 per cent.); (2) Hypotonic or hypertonic solutions of sodium chloride containing also potassium chloride 0·02 per cent. and calcium chloride 0·03 per cent. —these are subsequently termed hypotonic or hypertonic Ringer's solution; (3) Hypotonic or hypertonic Ringer's solution with the addition of 0·1 or 0·2 per cent. sodium sulphate (anhydrous). The solutions were fully oxygenated and were filtered before being put into the perfusion bottle. Frequently oxygen was also bubbled through the perfusing fluid in the bottle. The perfusion bottles were provided with a Mariotte tube.

2. *Physical.*—The greater part of the work which required analytical determinations of the materials used was carried out by means of the refractometer. The instrument used was of the Pulfrick type without water cooler. To enable the instrument to work with less than one drop of liquid, a small flat bottom tube was placed in the refractometer cup. Between the top of the prism and the bottom of the flat bottom tube there was a thin film of the liquid tested; in the tube was a little water with a thermometer. As neither the Pulfrick angles nor the corresponding indices convey much meaning in the present communication, all the results are returned as having a refractive index equal to a solution of sodium chloride of some special strength. As has been shown before,* the refractive index of solutions is proportional to their concentration. The refractive index of water on the particular instrument used is at 20° C. $\pm$ 1° = 67° 12·7′ $\pm$ 0·8′ with a probable error of one determination of $\pm$0·65′. The value of NaCl is 1′ = 0·0443 per cent. NaCl, and the determinations of strength of sodium

* B. Walter, 'Ann. Phys. Chem.,' vol. 38, p. 107; 'Journ. Chem. Soc.,' 1890, A, p. 202.

chloride between $\frac{1}{2}$ and 2 per cent. of pure NaCl showed a probable error of $\pm$ 0·02 per cent. NaCl in such solutions. The actual figures given in the communication must be considered as having that degree of error. Although other salts, as KCl, $CaCl_2$, were used, the amounts taken were too small to introduce any appreciable error on this account. Since the solutions used were very nearly solutions of one single chemical substance, the refractometer readings, like specific gravity, give the molecular concentration of the solution.

3. *Histological.*—Immediately after each experiment the kidneys were removed and placed in a fixing solution. This was generally alcohol, but in some cases formalin (10 per cent.) and in others Flemming's fluid was used. After being hardened the kidneys were embedded in paraffin and a series of sections was taken from the middle of each kidney. When the blood-vessels had been perfused with a mercuric salt and ammonium sulphide the sections were mounted unstained. In other cases stains were used, generally hæmatoxylin and eosin. In one case complete serial sections were made of the pair of kidneys.

Injections of the blood-vessels were also made as described below and serial sections prepared.

Results.

Histological.—The validity and significance of most of the experiments to be described rests upon the proof that the whole of the capillary network round the tubules normally receives blood both from the efferent vessels of the glomeruli (that is, by way of the arterial system) and from the renal portal vein. In order to demonstrate this we have injected a number of kidneys on the one hand from the aorta, after ligature of the renal portal veins, and on the other hand from the renal portal veins after occlusion of the arterial circulation. The injections were made from a perfusion bottle under a pressure approximately equivalent to the normal blood-pressure in the frog's kidney, namely 20–24 cm. of water for the arterial perfusion and 10–12 cm. of water for the venous perfusion. In all the experiments the venous outflow was unobstructed. The fluids used consisted of (*a*) Berlin blue and (*b*) carminate of ammonia in Ringer's solution. A few double injections were made, carmine by the arteries and Berlin blue by the renal portal vein. One gelatin double injection was also made, but in this case the pressure used was obtained by means of an injection syringe and pressure bottle and was higher than usual. It was found that whether the single injection was made by the artery or by the renal portal vein the whole of the intertubular capillary network appeared to be injected. In the case of the double injections the glomeruli and efferent vessels were filled with the

arterial injection fluid, whereas the intertubular capillaries showed, some the arterial fluid, some the venous, and some a mixture of both. We confirmed Beddard's observation that the perfusion of Berlin blue solutions at a low pressure through the renal portal vein leaves the glomeruli completely uninjected. We found, however, that if the venous perfusion were made under an abnormally high pressure (*e.g.* 35 cm. of water) the coloured solution eventually made its way into some, at least, of the glomeruli. It was further shown by arterial perfusion with Berlin blue that the glomeruli will withstand a perfusion pressure of at least 40 cm. of water.

Other evidence that the renal portal blood supplies the whole of the tubules was obtained by perfusing 1/10,000 mercuric chloride at 10 cm. pressure for three to five minutes through the renal portal vein, and then perfusing through the vein under the same pressure first saline solution for a few minutes and then a very weak solution of ammonium sulphide in saline solution. The whole of the tubules showed a deposit of mercuric sulphide while the glomeruli remained free from it in the vast majority of cases. It seems clear, therefore, that a poison reaching the kidney by way of the renal portal vein will come in contact with the whole of the tubules and yet leave the glomeruli practically or quite intact. The only risk is that a diffusible poison, if not quickly rendered inert or washed out of the kidney, may gradually reach the glomeruli by direct diffusion. This risk is minimised by having a simultaneous arterial perfusion and by using the poison in a concentration which is just adequate to kill the tubules when brought into immediate contact with them. Whether the arterial blood supply alone provides the tubules with a sufficiency of oxygen has yet to be determined by experiments on the living animal. It is known, however, that the venous supply alone will maintain their nutrition in the living frog provided that the frog is kept in an atmosphere of oxygen. There can be little doubt that in these experiments, in which the perfusing fluid was fully oxygenated, both the supply of oxygen to the tubules in a venous perfusion and that to the glomeruli in an arterial perfusion were amply sufficient to maintain their vitality so long as the rate of perfusion remained normal.

Experimental.—In most of the experiments to be described the perfusion was made with normal or hypotonic Ringer's solution, and the experiments made with hypertonic Ringer's solution will only be referred to incidentally.

(1) *The Normal Kidney.*—The rate of arterial perfusion varies considerably in different experiments, doubtless as a result of the varying tone of the glomerular vessels, and more particularly the efferent vessels; it is apt also to decrease in the course of a single experiment. Since the oxygen supply

to the kidneys varies directly with the perfusion rate, a slow perfusion leads to an inadequate oxygen supply to the glomeruli or tubules.

The amount of fluid escaping from the renal veins on an arterial perfusion alone varied from 20 to 60 c.c. per hour in different experiments; an average rate was about 30 c.c. per hour. On a venous perfusion alone the rate of perfusion was more constant and averaged 60–70 c.c. per hour.

Flow of Urine.—The amount of urine obtained from the normal kidneys on an arterial perfusion alone varies directly with the rate of perfusion, and under favourable circumstances as much as 1·5 c.c. may be obtained in less than an hour. The concentration of the urine is almost always notably less than that of the perfusing fluid when the latter is hypotonic Ringer's solution; if the kidneys are perfused with normal Ringer's solution the urine may be isotonic with, but is usually hypotonic to, the perfusing fluid.

The urine obtained on a simultaneous arterial and venous perfusion does not, so far as we could determine, differ in amount from that obtained on an arterial perfusion alone; a simultaneous arterial and venous perfusion, however, seems to be more conducive to the formation of a very dilute urine than is arterial perfusion alone.

Table I.—Urine from Living and Dead Kidneys.

Experiment.	Concentration.		
	Perfusing fluid.	Urine from normal kidneys.	Urine from dead kidneys.
		per cent. per cent.	per cent. per cent.
1	0·59 per cent. NaCl	(*a*) 0·42 (*b*) 0·46	
2	0·57 „ „	(*a*) 0·40 (*b*) 0·38	
3	0·55 „ „	(*a*) 0·33 (*b*) 0·40	
4	0·53 „ „	0·25	
5	0·42 „ „	0·30	0·40
6	0·72 „ „	(*a*) 0·55 (*b*) 0·49	
7	0·83 „ „	(*a*) 0·73 (*b*) 0·68	(*a*) 0·83 (*b*) 0·83

The letters (*a*) and (*b*) refer to successive samples of urine.

In Experiment 7 the perfusing fluid contained 0·1 per cent. Na_2SO_4; in the others the perfusing fluid was simply normal or hypotonic Ringer's solution.

On a venous perfusion alone no urine was secreted with any of the perfusing fluids used. In some of these experiments the arterial circulation was excluded by tying the aortic bulb at the outset and allowing the glomeruli to become infarcted; in others, the glomeruli had previously been perfused with Ringer's solution and the arterial perfusion shut off.

(2) *The Dead Kidney.*—The vitality of the kidney was destroyed by perfusing it through the aorta either with weak (1/10,000) corrosive sublimate or with boiled Ringer's solution. After the former procedure the arterial perfusion with Ringer's solution was resumed under normal pressure. The rate of perfusion and the amount of urine obtained were always much less than in the normal kidney, and sometimes, with a very slow perfusion rate, the flow of urine entirely ceased. The urine was usually isotonic with, but occasionally hypertonic to, the perfusing fluid, the latter only in experiments in which the formation of urine was extremely slow and scanty.

(3) Since the urine obtained in all these experiments comes solely from the glomeruli (the tubules secrete no urine), it is natural to suppose that the difference in the character of the urine formed by the intact and dead kidneys respectively depends upon one of two causes. On the one hand, the glomeruli may normally form by filtration a urine which is isotonic with the perfusing fluid, and the absorption of salt may be effected by the tubules as the glomerular filtrate passes along them. On the other hand, the tubules may possess no absorptive power for sodium chloride or other salts, and the glomeruli may possess the capacity to secrete a hypotonic urine. In attempting to decide between these two possibilities, two methods have been used.

(*a*) The tubules were poisoned by perfusing 1/10,000 mercuric chloride through the renal portal vein for three to five minutes, and then Ringer's solution was perfused for a few minutes through the renal portal veins, to wash away the mercury in the blood-vessels. The arterial perfusion of oxygenated Ringer was maintained throughout the experiment. The urine obtained both before and after the poisoning of the tubules was examined, and at the end of the experiment the mercury was fixed in the tubule cells by perfusing dilute ammonium sulphide through the renal portal veins, and the kidneys were examined histologically. It was found in most of the experiments that the glomeruli remained free from mercury, and that mercuric sulphide was present in the whole of the tubules. This was also the case in control experiments in which the mercury was fixed by ammonium sulphide immediately after it had been perfused through the renal portal vessels. Experiments in which mercury was present in the glomeruli were rejected. The following protocol illustrates the character of these experiments:—

Protocol I.—Pithed Male Frog. Cannulæ in left aorta, origin of vena cava, anterior abdominal vein and ureters. Hind legs tied off. Mesenteric artery and right aorta ligatured and testes removed.

	Time.	Fluid escaping by v. cava.	Urine.	Concentration of urine.
		c.c.		
Simultaneous arterial and venous perfusion oxyg. Ringer's solution.	3.15–3.30	22	3.10–3.30 R. K. 0·1 c.c.	0·33 per cent. NaCl.
	3.30–3.45	27	3.30–3.40 R. K. 0·2 „	0·32 „ „
			L. K. 0·2 „	0·29 „ „
	3.45–4.0	21	3.40–3.57 R. K. 0·2 „	0·41 „ „
			L. K. 0·2 „	0·40 „ „
3.58–4.4. Perfused 1/10,000 $HgCl_2$ through renal portal veins. Arterial perfusion maintained. Then perfused Ringer's solution for 5 minutes through renal portal veins, to wash the mercuric chloride out of the vessels.				
Simultaneous perfusion, art. oxyg., venous non-oxyg., but containing 0·1 p. c. caffein. A. press. 24 cm. V. 12 cm. Ringer = 0·53 per cent. NaCl	4.0–4.30	43	4.0–4.30 R. K. 0·1 c.c.	0·53 per cent. NaCl
			L. K. 0·1 „	0·52 „ „
	4.30–5.0	48		
Finally perfused weak ammonium sulphide through renal portal vein. Kidneys cut in serial section; no mercury observed histologically in the glomeruli.				

The venous perfusion of corrosive sublimate causes considerable vaso-constriction, and the efferent vessel from the glomerulus which ends in the tubular capillary network appears to be particularly affected. This, at least, is our interpretation of the extremely slow perfusion through the glomeruli which is met with under these circumstances and which is associated with a lessened flow of urine. We have attempted to overcome this vaso-constriction by perfusing through the renal portal veins Ringer's solution containing a trace of acetic acid or 0·1 per cent. caffein sodium benzoate, but without complete success. We have obtained, however, a rate of perfusion through the glomeruli which was adequate to maintain their vitality, and in some experiments was for a time equivalent to that of the normal kidney, although it eventually became very slow. The employment of 1/10,000 mercuric cyanide caused less vaso-constriction, but also less definite histological evidence of the complete poisoning of the tubules. The results of a number of these experiments are shown in Table II.

Table II.—Effect of Poisoning the Tubules with Corrosive Sublimate. The perfusing fluid was in all cases hypotonic Ringer's solution.

Exp.	Concentration of urine.		Concentration of perfusing fluid.	Histological result.
	Normal kidneys.	Poisoned kidneys.		
	per cent.	per cent.		
1	0·33, 0·29, 0·40	0·53, 0·52	0·53 per cent. NaCl	Glomeruli intact, tubules all show Hg.
2	0·48, 0·43	0·60	0·58 „ „	
3	0·30	0·40	0·42 „ „	
4	0·47, 0·40	0·51	0·57 „ „	

Different figures represent separate samples of urine.

(*b*) The kidneys were first perfused with oxygenated Ringer's solution simultaneously by the aorta and renal portal veins. Then the glomeruli were perfused with boiled Ringer's solution in order to kill them, while the tubules were still receiving by the veins an adequate supply of oxygen. A typical experiment is shown in the following protocol:—

Protocol II.—Pithed Male Frog. Cannulæ in left aorta, anterior abdominal vein, inferior vena cava and ureters. Mesenteric artery and right aorta ligatured. Hind legs tied off. Simultaneous arterial and venous perfusion. Arterial pressure 24 cm., venous 12 cm. Molecular concentration of Ringer's solution (refractometer) = 0·55 per cent. NaCl. Ringer's solution contained 0·5 per cent. NaCl, 0·02 per cent. KCl, 0·03 per cent $CaCl_2$ in distilled water.

Time.	Escape from vena cava.	Urine.	Concentration of urine.
	c.c.		
11.47–12.4	31	11.40–12.10 R. K. 0·1 c.c.	0·40 per cent. NaCl
		L. K. 0·15 „	0·46 „ „
12.4–12.28	51		
12.28–12.47	37	12.10–12.48 R. K. 0·15 „	0·33 „ „
		L. K. 0·15 „	0·34 „ „
12.47–1.17	33	12.48–2.15, both kidneys 0·1 c.c.	0·36 „ „
1.17–1.47	27		
1.47–2.17	23		
2.17–2.47	25		

12.50 onwards. Glomeruli perfused with boiled Ringer's solution.

It will be noticed that the cutting off of the oxygen supply to the glomeruli lessened the rate of arterial perfusion and also the amount of urine formed. The general result of a number of such experiments is shown in the following table:—

Table III.

Expt.	Concentration of urine.		Concentration perfusing fluid.
	Normal kidney.	Glomeruli killed.	
1	(*a*) 0·48 per cent. NaCl (*b*) 0·45 „ „	(*a*) 0·45 per cent. NaCl (*b*) 0·45 „ „	0·56 per cent. NaCl
2	(*a*) 0·43 „ „ (*b*) 0·34 „ „ (*c*) 0·33 „ „	0·36 „ „	0·55 „ „
3	(*a*) 0·48 „ „ (*b*) 0·38 „ „	(*a*) 0·42 „ „ (*b*) 0·43 „ „	0·50 „ „
4	(*a*) 0·46 „ „ (*b*) 0·36 „ „	(*a*) 0·48 „ „ (*b*) 0·48 „ „	0·53 „ „

In all the experiments the perfusing fluid contained sodium chloride plus 0·02 per cent. KCl and 0·03 per cent. $CaCl_2$. The letters (*a*) and (*b*) refer to separate samples of urine.

(*c*) *Caffein.*—Barcroft and Straub have shown that caffein sodium benzoate greatly diminishes the consumption of oxygen by the mammalian kidney, and they used it for the purpose of poisoning the renal tubules. We have carried out a number of experiments to determine the action of caffein on the frog's kidney. In our early experiments 1 per cent. caffein sodium benzoate was perfused through the renal portal veins for five minutes, the arterial perfusion of oxygenated Ringer's solution being simultaneously maintained; the collection and examination of the urine was not begun until from one to two hours after the perfusion of the caffein, and it was found to be hypotonic to the perfusing fluid. At that time we believed that caffein (in the dose given) permanently poisoned the tubules, and we therefore regarded the glomeruli as capable of secreting a hypotonic urine. Further experiment seems to show, however, that the poisoning effect of caffein is merely temporary. The immediate effect is to render the urine isotonic with the

Table IV.—Action of Caffein Sodium Benzoate.

Expt.	Concentration of fluid in terms of NaCl.			
	Perfusing fluid.	Normal urine.	Urine after poisoning tubules with caffein	
			Immediately after.	1–2 hours after.
	per cent.	per cent.	per cent.	per cent.
1	0·53	0·25	0·54	—
2	0·55	0·40	0·55	—
3	0·51	—	—	0·22, 0·30

perfusing fluid, but this effect gradually passes off and, unless more caffein is injected, the urine once more becomes hypotonic to the perfusing fluid. We found that if 0·1 per cent. caffein were continuously perfused through the renal portal veins the urine remained isotonic with the arterial perfusing fluid throughout the experiment. Unfortunately it is impossible to trace the caffein histologically into the tubule cells, and we do not know whether it attacks the whole length of the tubules or not. We regard these results, therefore, as merely subsidiary to and confirmatory of those in which the tubules were poisoned with mercury.

The formation of a urine which is hypertonic to the perfusing fluid has occasionally been observed after poisoning the tubules with corrosive sublimate and even in the dead kidney. It occurs only when the formation of urine is extremely slow ; we are not yet satisfied as to its significance.

Summary and Conclusions.

When the frog's kidneys are perfused through the aorta and the renal portal veins with oxygenated normal or hypotonic Ringer's solution, the urine formed is hypotonic to the perfusing fluid and is derived entirely from the glomeruli, since the tubules secrete no urine under these circumstances. When the tubules are poisoned with corrosive sublimate or (temporarily) with caffein, the urine becomes isotonic with the perfusing fluid. On the contrary, if the glomeruli are killed by the arterial perfusion of boiled Ringer's solution, while the tubules still receive an adequate supply of oxygen through the renal portal veins, the urine formed continues to be more dilute than the perfusing fluid. These results suggest, first, that the glomeruli form by filtration a urine isotonic with the perfusing fluid, and, secondly, that during the passage of the glomerular filtrate down the tubules sodium chloride is absorbed by them. Whether any water is also absorbed we do not know.

The expenses of this research have been defrayed by a grant from the Government Grant Committee of the Royal Society.

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